Biosignal-Based Computing by AHL Induced Synthetic Gene Regulatory Networks

From an *in vivo* Flip-Flop Implementation to Programmable Computing Agents

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www.zbi-saar.de

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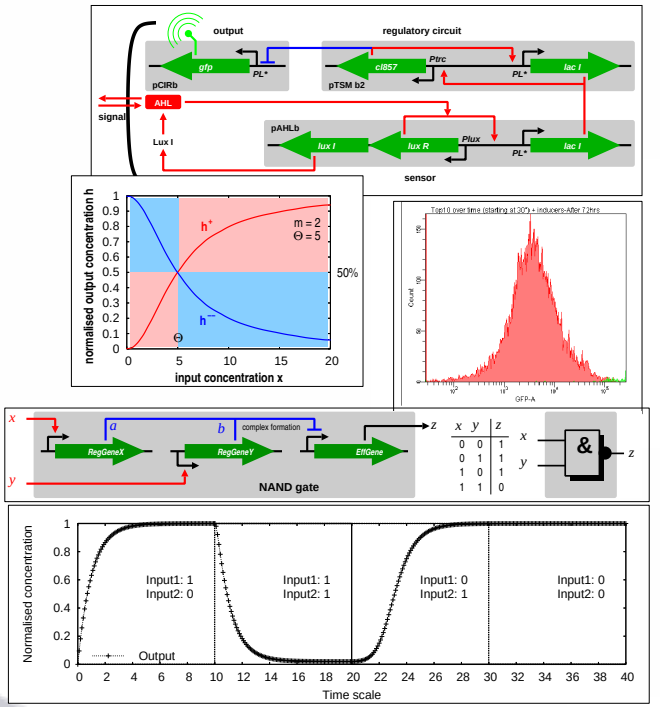
Outline

Biosignal-Based Computing by AHL Induced Synthetic GRNs

1. **Introduction**
2. **Gene Regulatory Networks (GRNs)**
3. **Hill kinetics**
4. **Case study: computational units**
5. **RS flip-flop wetlab implementation in *Vibrio fischeri***
6. **Synthetic GRN for knapsack problem solution**
7. **Conclusions, further work**



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Evolving Cell Signalling Networks *in silico*

European interdisciplinary research project

- University of Birmingham (Computer Science)
- TU Eindhoven (Biomedical Engineering)
- Dublin City University (Artificial Life Lab)
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SIXTH FRAMEWORK PROGRAMME

Objectives

- Study computational properties of CSNs/GRNs
- Develop new ways to model and predict real CSNs/GRNs
- Gain new theoretical perspectives on real CSNs/GRNs

Collaboration partner for *in vivo* studies

- BIOTEC at Dresden University of Technology

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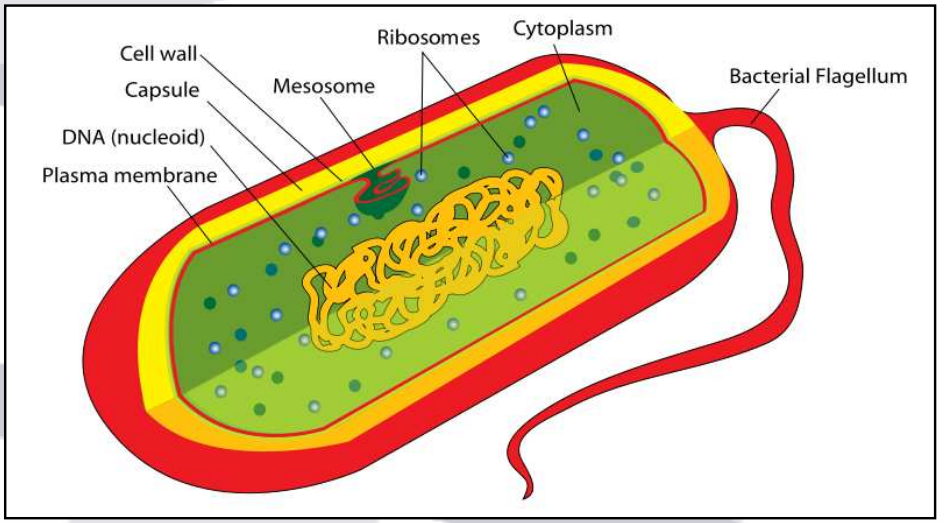
Motivation and Intention

- Computing *in vivo*
- Synthetic/evolutionary predefined computational units
- Implementation in micro-organisms
- Vision: potentially miniaturised, robust, reliable, energy-efficient and bio-compatible hardware

$\Longrightarrow$ **Construction, programming, applicability?**



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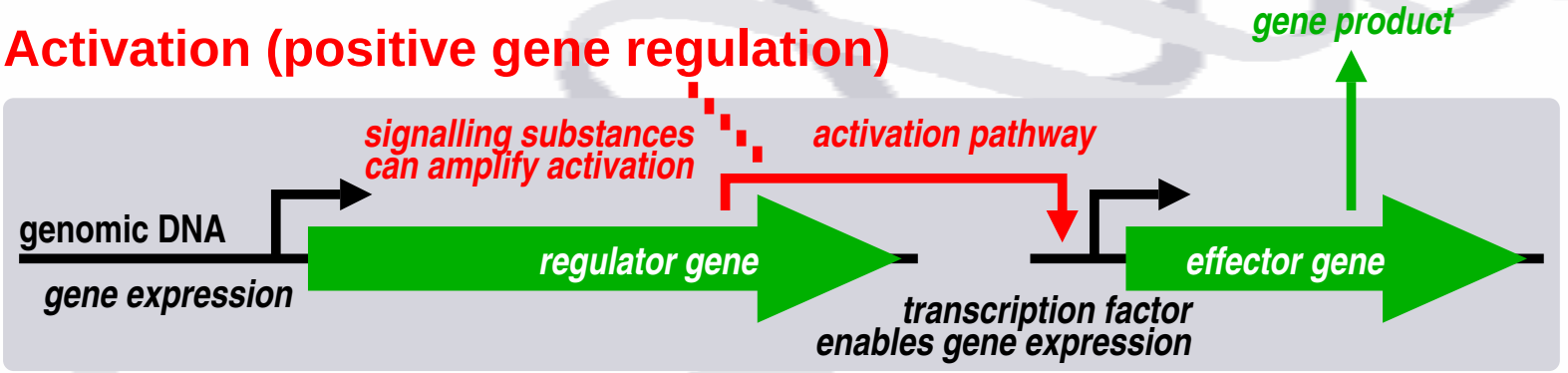
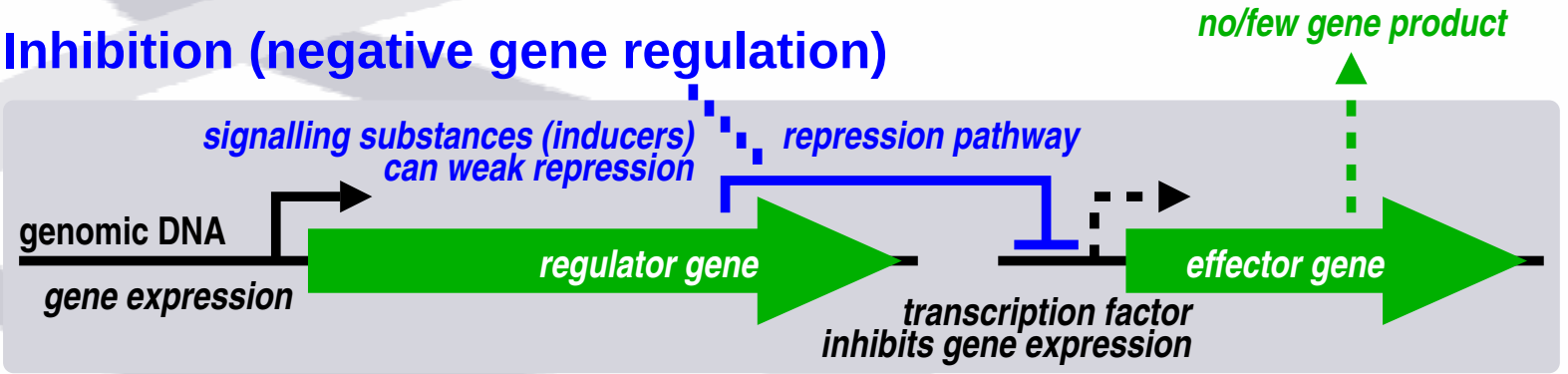
Biological Principles of Gene Regulation

Intercellular Information Processing of Spatial Globality within Organisms

Activation (positive gene regulation)



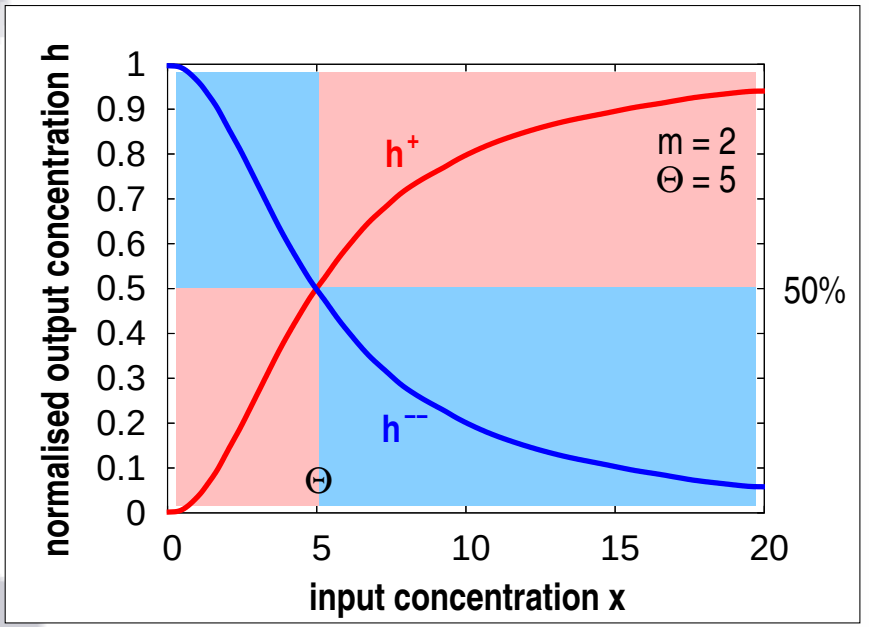
Inhibition (negative gene regulation)



Feedback loops: gene products can act as transcription factors and signalling substances forming gene regulatory networks

Hill Kinetics – Sigmoid-Shaped Threshold Functions

- Model cooperative and competitive aspects of interacting gene regulatory units dynamically and quantitatively
- Homogeneous and analytic
- Formulate relative intensity of gene regulations by **sigmoid-shaped threshold functions**
  $h^+, h^- : \mathbb{R} \times \mathbb{R} \times \mathbb{N} \to \mathbb{R}$
- $x \geq 0$: input concentration of **transcription factor** activating/inhibiting gene expression
- $\Theta > 0$: threshold (50% level)
- $m \in \mathbb{N}_+$: degree of regulation



activation (upregulation) $h^+(x, \Theta, m) = \frac{x^m}{x^m + \Theta^m}$

inhibition (downregulation) $h^-(x, \Theta, m) = 1 - h^-(x, \Theta, m)$

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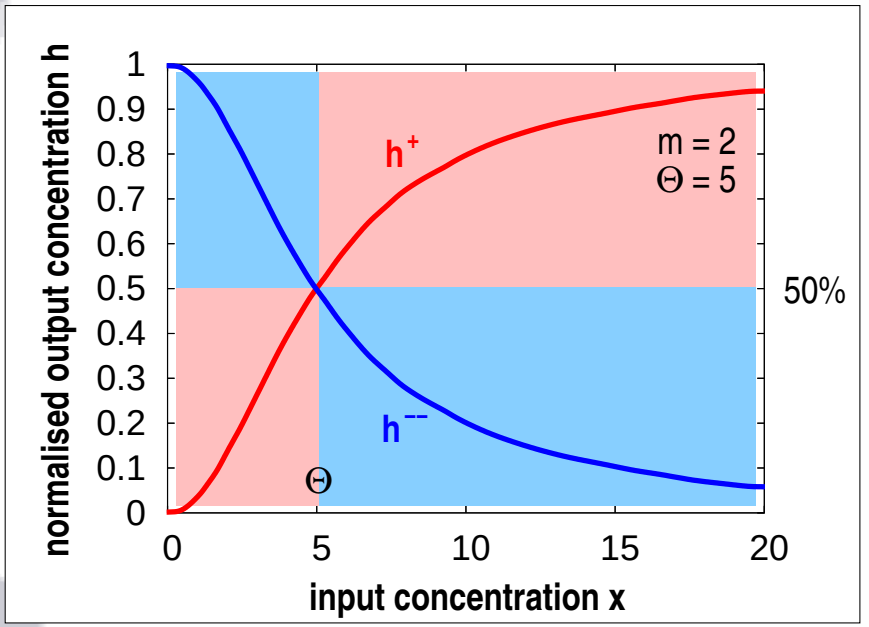

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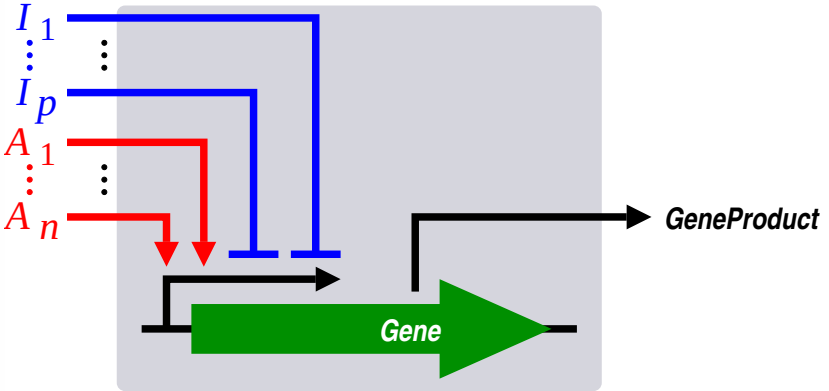
Hill Kinetics – Modelling Dynamical Network Behaviour

- Several interacting (competing) transcription factors influence gene expression
- Activators A_i, inhibitors I_j and proportional factor $c_1 > 0$: determine **production rate** of a **gene product**
- Additional assumption of linear spontaneous **decay rate** $c_2 \cdot [GeneProduct]$ with $c_2 > 0$
- Differential equation for corresponding gene product:



$$\begin{aligned}\frac{d[GeneProduct]}{dt} &= ProductionRate - c_2[GeneProduct] \\ &= c_1 \cdot h^+(A_1, \Theta_{A_1}, m) \cdot \ldots \cdot h^+(A_n, \Theta_{A_n}, m) \cdot \\ &\quad (1 - h^+(I_1, \Theta_{I_1}, m) \cdot \ldots \cdot h^+(I_p, \Theta_{I_p}, m)) \\ &\quad - c_2 \cdot [GeneProduct]\end{aligned}$$

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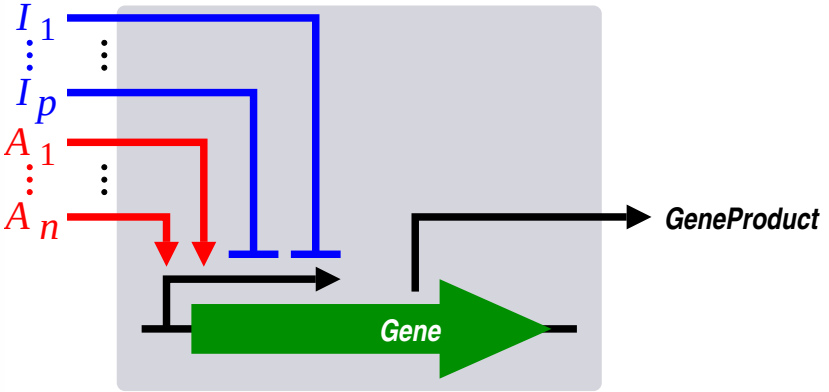

$$\begin{aligned}\frac{\mathrm{d}\,[GeneProduct]}{\mathrm{d}t} &= ProductionRate - c_2[GeneProduct] \\ &= c_1 \cdot \mathrm{h}^+(A_1, \Theta_{A_1}, m) \cdot \ldots \cdot \mathrm{h}^+(A_n, \Theta_{A_n}, m) \cdot \\ &\quad (1 - \mathrm{h}^+(I_1, \Theta_{I_1}, m) \cdot \ldots \cdot \mathrm{h}^+(I_p, \Theta_{I_p}, m)) \\ &\quad - c_2 \cdot [GeneProduct]\end{aligned}$$

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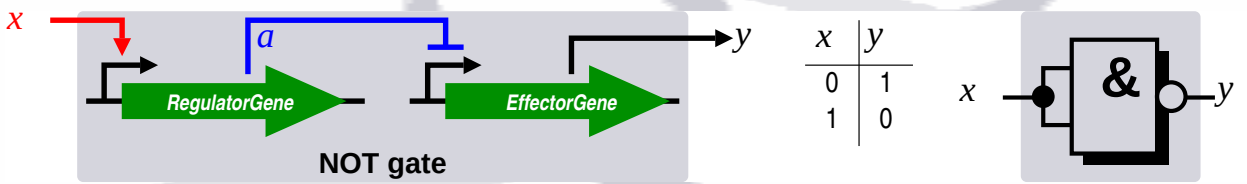
Case Study: Inverter

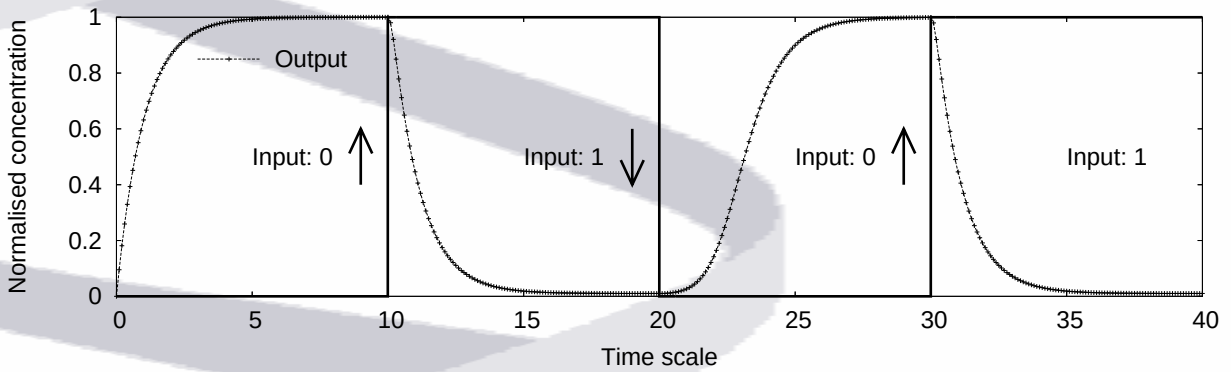
Input: concentration levels of transcription factor x

Output: concentration level of gene product y



Dynamical behaviour depicted for $m = 2$, $\Theta_j = 0.1$, $j \in \{x, a\}$,

$$a(0) = 0,\ y(0) = 0,\ x(t) = \begin{cases} 0 & \text{for } 0 \le t < 10;\ 20 \le t < 30 \\ 1 & \text{for } 10 \le t < 20;\ 30 \le t < 40 \end{cases}$$



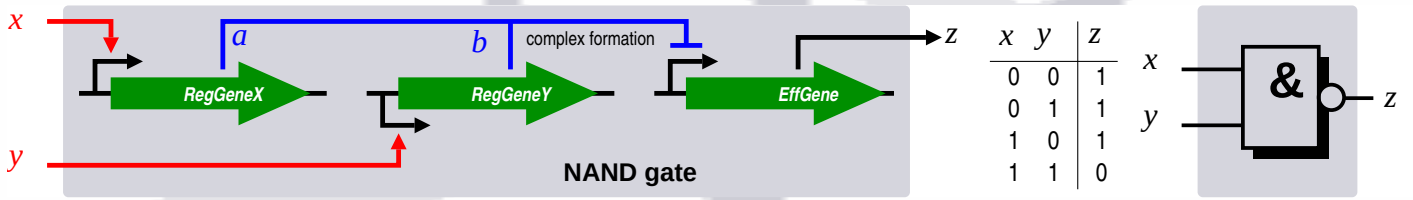
$$\dot{a} = \mathrm{h}^{+}(x, \Theta_x, m) - a$$

$$\dot{y} = \mathrm{h}^{-}(a, \Theta_a, m) - y$$

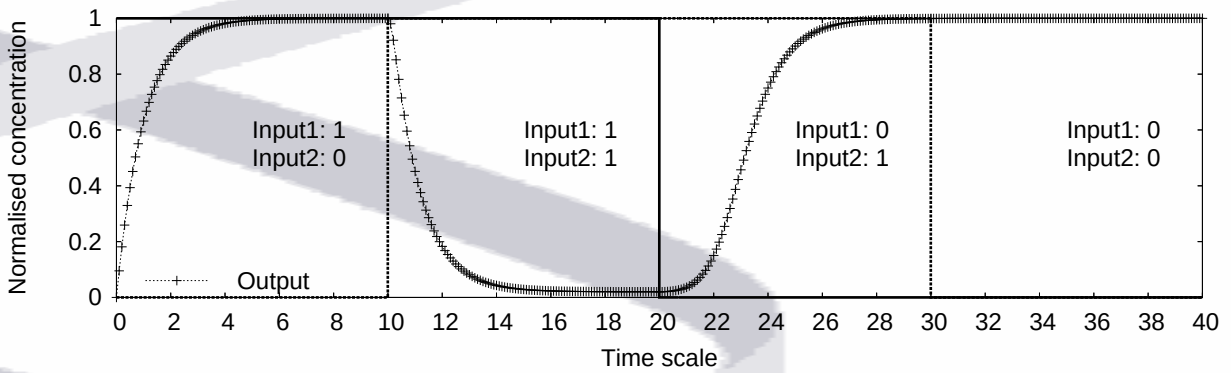
Case Study: NAND Gate

Input: concentration levels of transcription factors x (inp.1), y (inp.2)

Output: concentration level of gene product z



Dynamical behaviour depicted for $m = 2$, $\Theta_j = 0.1$, $j \in \{x, y, a, b\}$

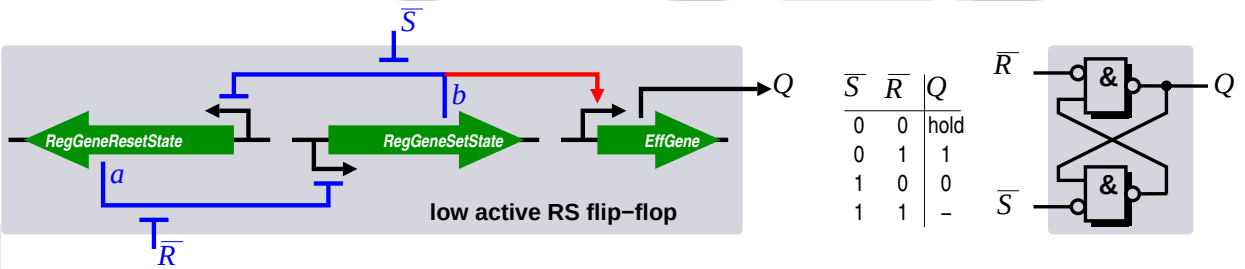


$$\dot{a} = \mathrm{h}^+(x, \Theta_x, m) - a$$
$$\dot{b} = \mathrm{h}^+(y, \Theta_y, m) - b$$
$$\dot{z} = 1 - \mathrm{h}^+(a, \Theta_a, m) \cdot \mathrm{h}^+(b, \Theta_b, m) - z$$

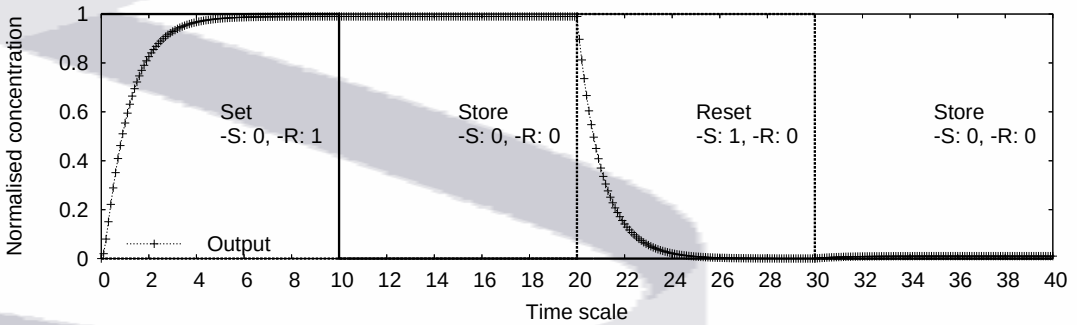
Case Study: RS Flip-Flop

Input: concentration levels of transcription factors $\overline{S}, \overline{R}$

Output: concentration level of gene product Q



Dynamical behaviour depicted for $m = 2$, $\Theta_j = 0.1$, $j \in \{a, b, \overline{R}, \overline{S}\}$



$$\dot{a} = 1 - \mathrm{h}^+(b, \Theta_b, m) \cdot \mathrm{h}^-(\overline{S}, \Theta_{\overline{S}}, m) - a$$

$$\dot{b} = 1 - \mathrm{h}^+(a, \Theta_a, m) \cdot \mathrm{h}^-(\overline{R}, \Theta_{\overline{R}}, m) - b$$

$$\dot{Q} = \mathrm{h}^+(b, \Theta_b, m) \cdot \mathrm{h}^-(\overline{S}, \Theta_{\overline{S}}, m) - Q$$

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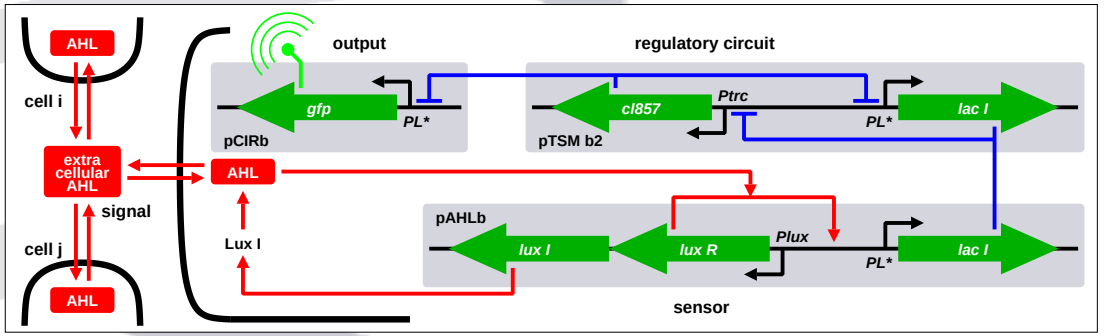
Quorum Sensing via AHL

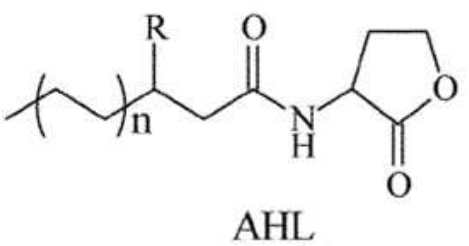
Quorum sensing (autoinduction)

- Intercellular communication between bacteria
- Regulation of gene expression based on bacteria-population density, e.g. in *Vibrio fischeri*



AHL (N-acyl homoserine lactone)

- Signal molecule
- Autoinducer
- Produced and released by bacterial cells
- Critical concentration $\longrightarrow$ activation of gene expression



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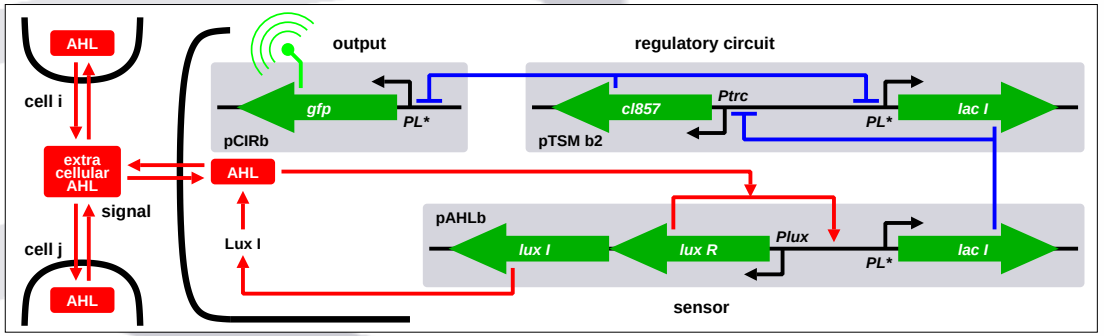

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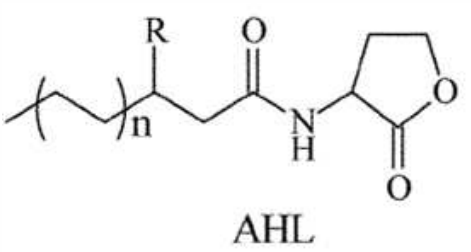
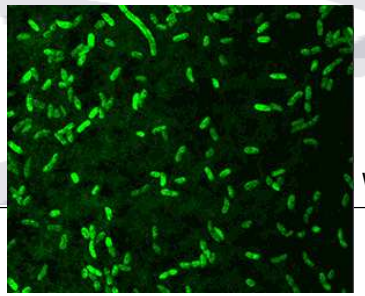


AHL

Bioluminescence in *Vibrio fischeri*

Enzyme catalysed reaction emitting photons



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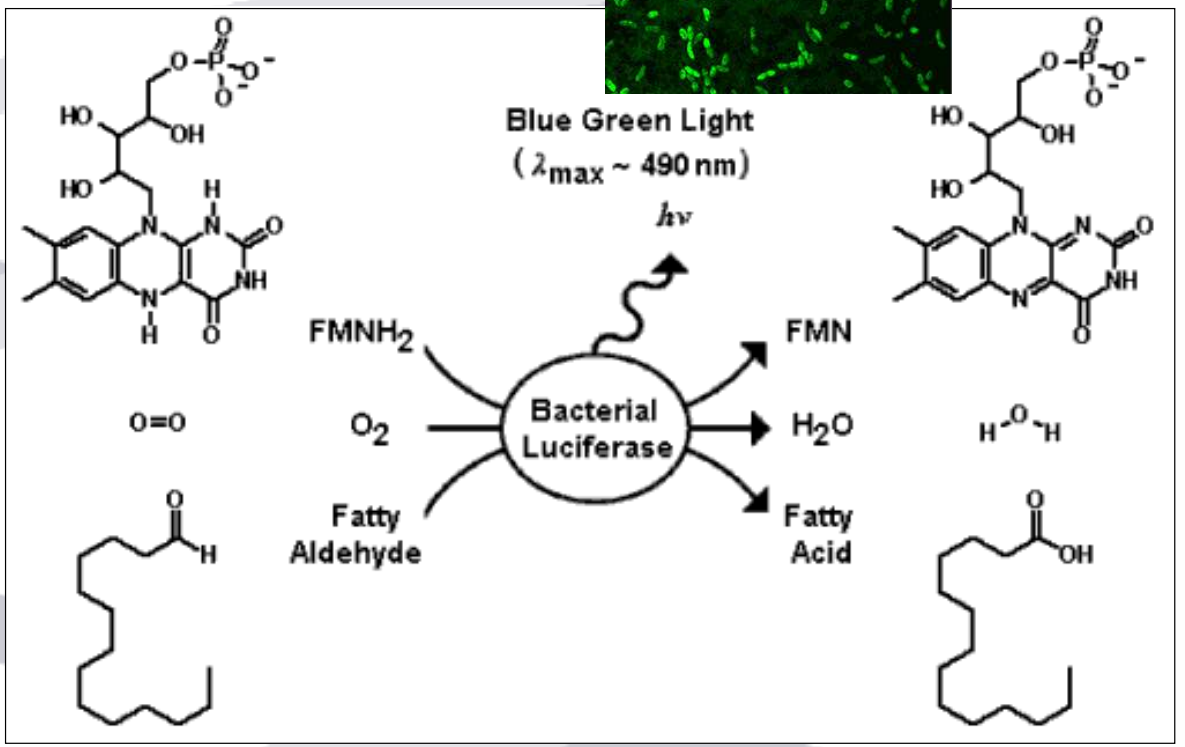
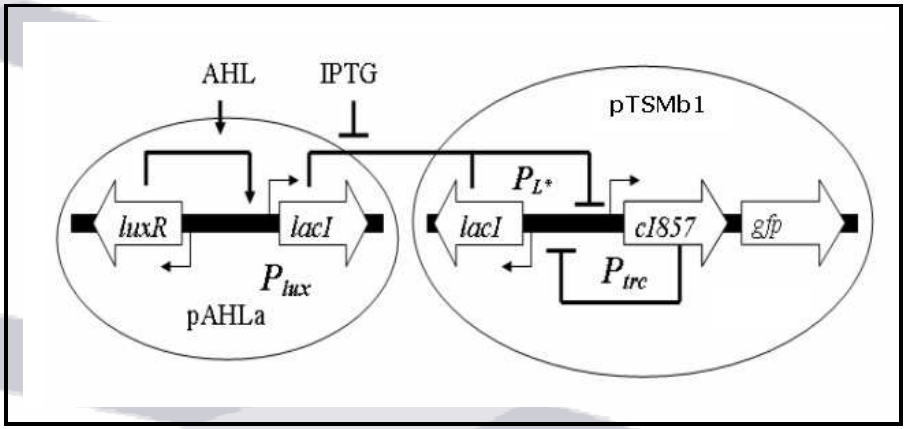


$FMNH_2$: flavin mononucleotide (luciferin)

Wetlab Implementation of GRN-Based RS Flip-Flop

Experimental Setup

- *in vivo* system (bistable toggle switch in *Vibrio fischeri*) mimics RS flip-flop
- Encoding of all genes using two constructed plasmids
- Quantification of its performance using flow cytometry
- Presence or absence of inducers AHL and IPTG acts as input signals, green fluorescent protein (*gfp*) as output



Collaboration with S. Hayat, at this time Dresden University of Technology, BIOTEC laboratories.
Thanks to J.J. Collins, W. Pompe, G. Rödel, K. Ostermann, L. Brusch for their support.

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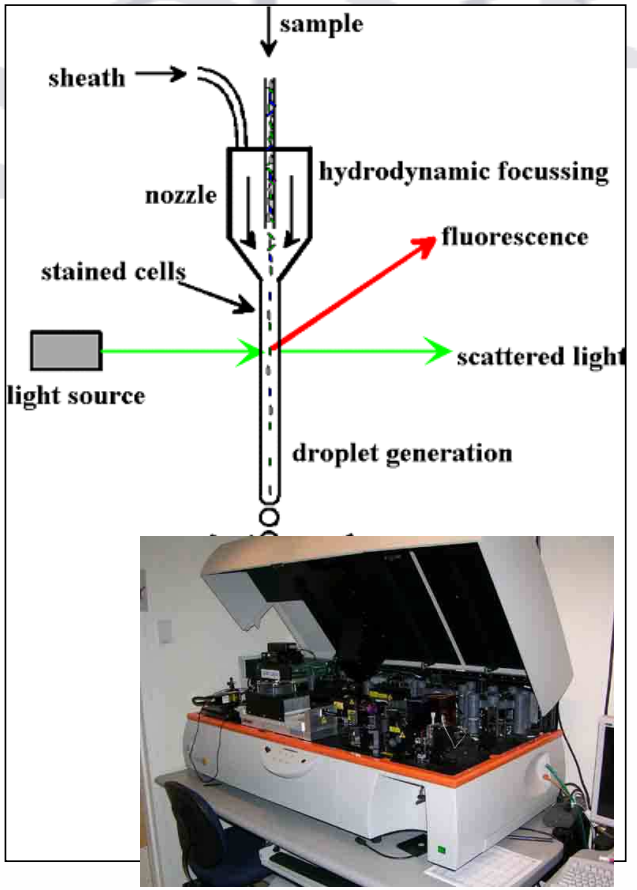
Flow Cytometry

- Technique for counting, examining, and sorting microscopic particles
- Particles focused in fluid stream
- Measuring point surrounded by array of laser detectors emitting light beam
- Each passing particle scatters light
- Fluorescent chemicals within particles emit light at lower frequency
- Fluctuation of brightness analysed at each detector $\longrightarrow$ particle count
- Quantification of *gfp* amount
- Cytometer used for experimental studies: Becton Dickinson LSR II (488nm laser)



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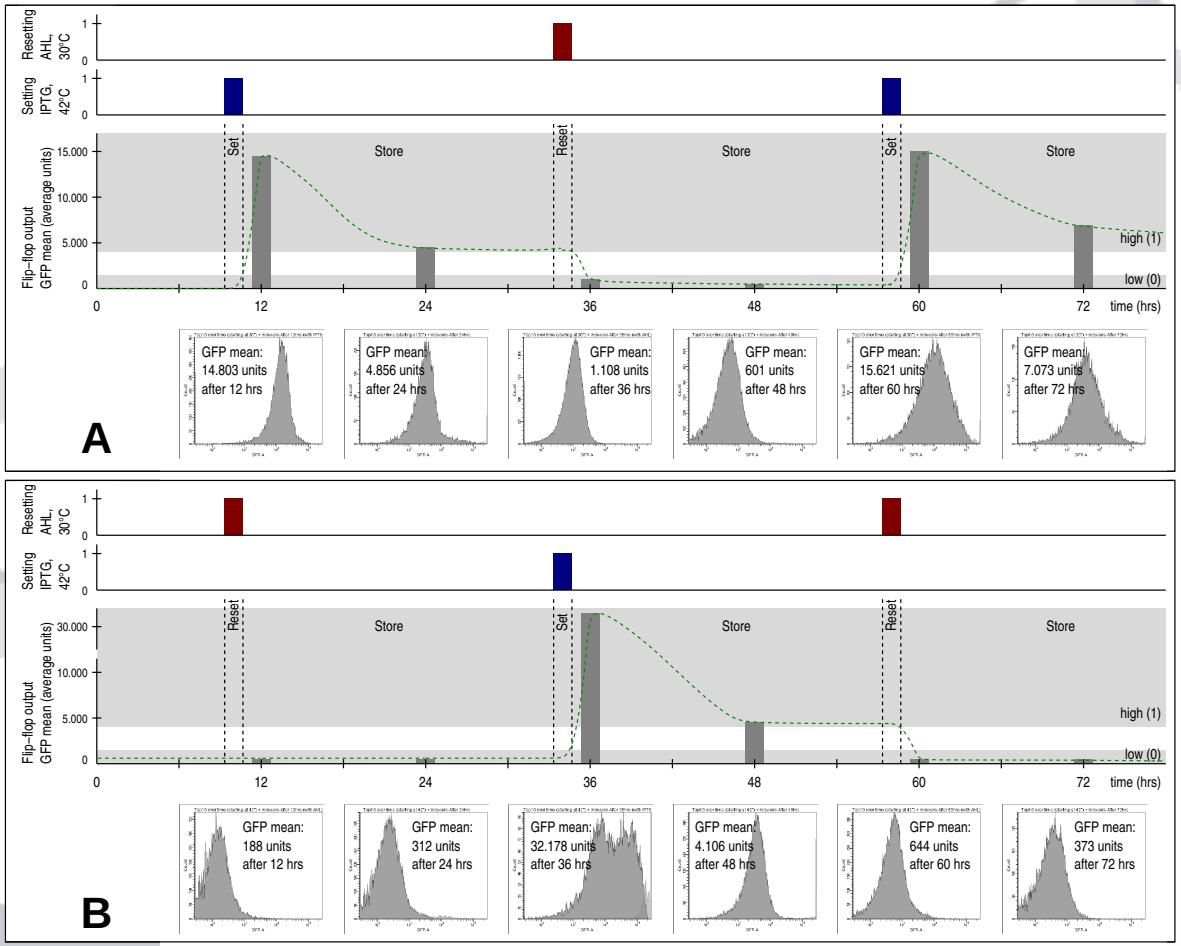
Wetlab Experimental Results



Repeated activation and deactivation of the toggle switch based on inducers and temperature. Temperature was switched every 24 hours. Cells were incubated with inducers for 12 hours, followed by growth for 12 hours without inducers, initially kept at 30°C (**A**) and 42°C (**B**). The cells successfully switched states thrice.

Collaboration with S. Hayat, at this time Dresden University of Technology, BIOTEC laboratories.
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Knapsack Problem

NP-complete, exponential need of resources for exact solution

Problem definition

There are n nat. numbers $a_1, \ldots, a_n$ and reference number $b \in \mathbb{N}$

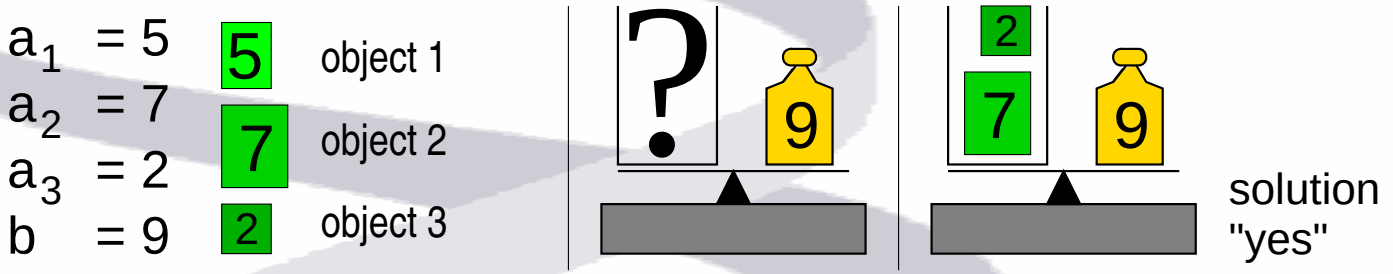
Is there a subset $I \subseteq \{1, \ldots, n\}$ with $\sum_{i \in I} a_i = b$?

Explanation

$a_1, \ldots, a_n$: **weights** of **objects** $1, \ldots, n$.

Is there a possibility to pack a selection of these objects into the knapsack and to meet the overall weight b exactly?

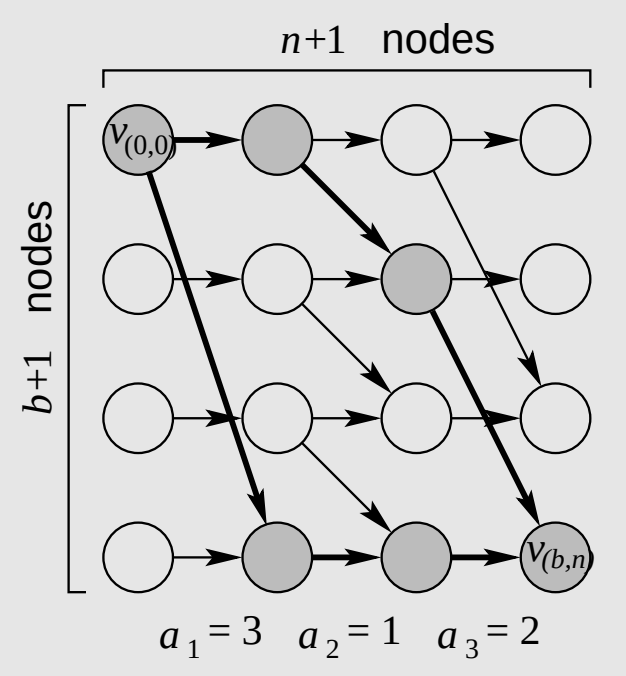
Example

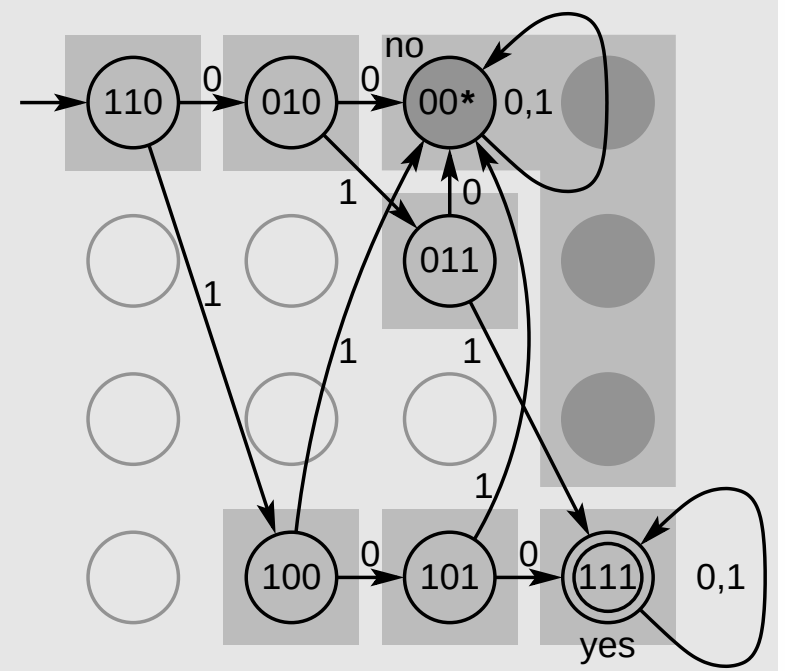


Solution to the Knapsack Problem: Strategy

Dynamic programming approach $\longrightarrow$ finite automaton

Example instance: $n = 3, a_1 = 3, a_2 = 1, a_3 = 2, b = 3$





- finite automaton $\longrightarrow$ circuit
  (based on NAND gates, RS-FFs, clock generator)
- circuit $\longrightarrow$ artificial GRN
- artificial GRN $\longrightarrow$ dynamical simulation (Hill kinetics)

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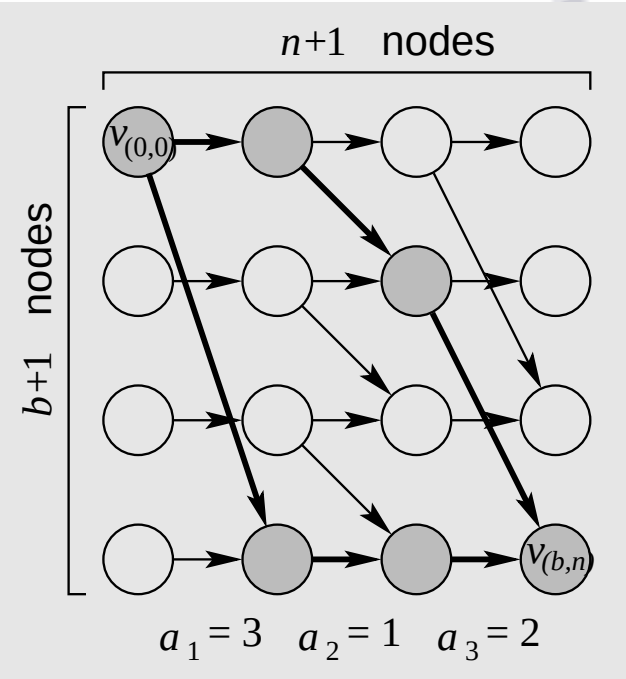

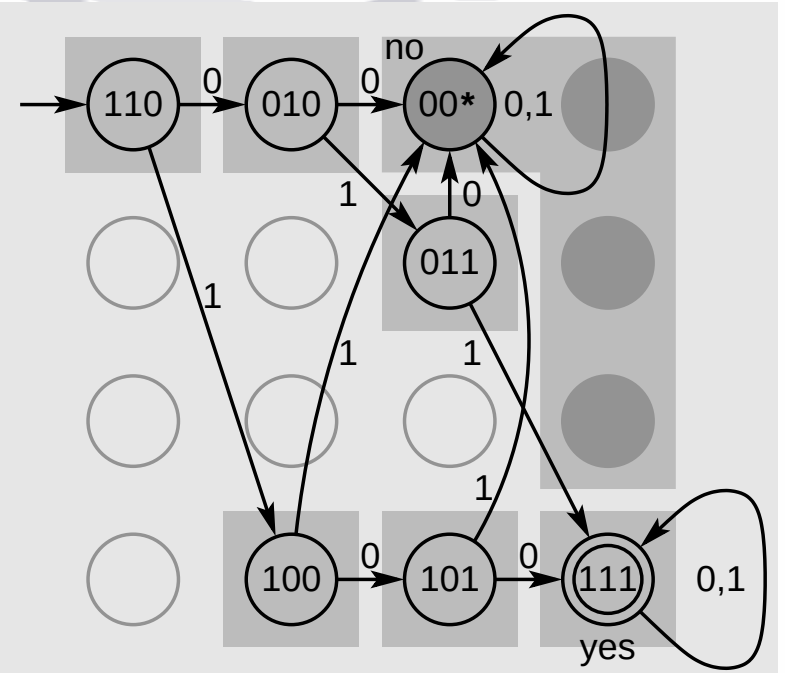

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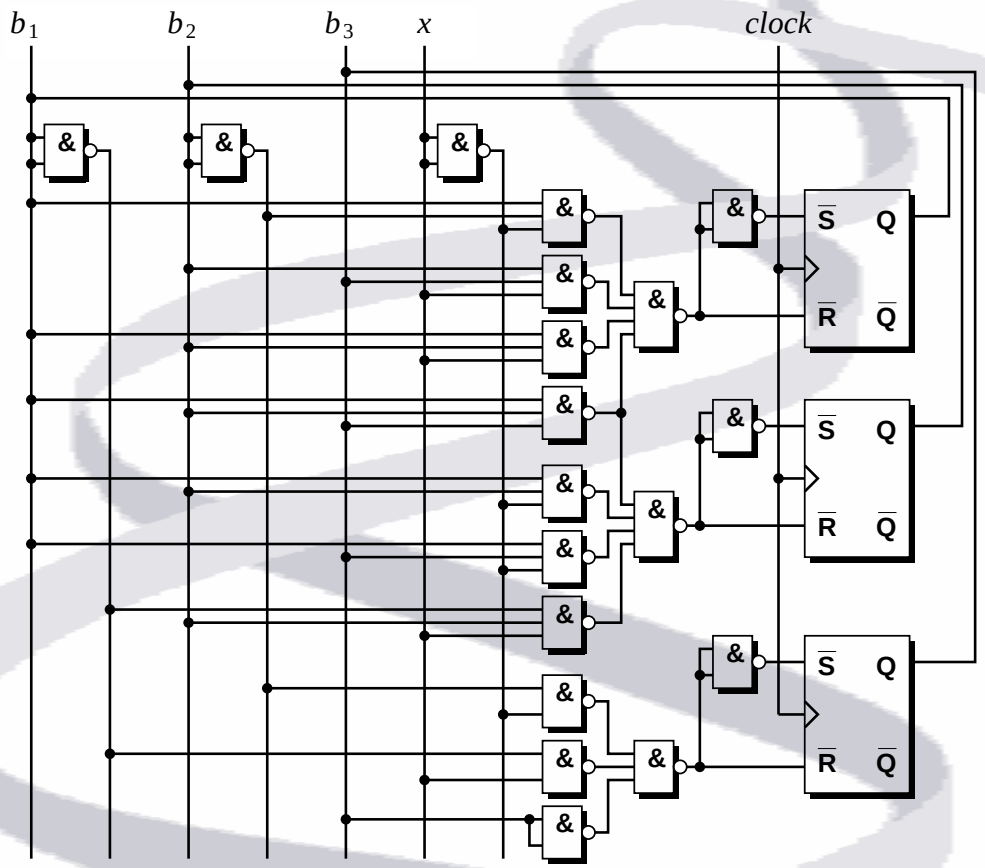
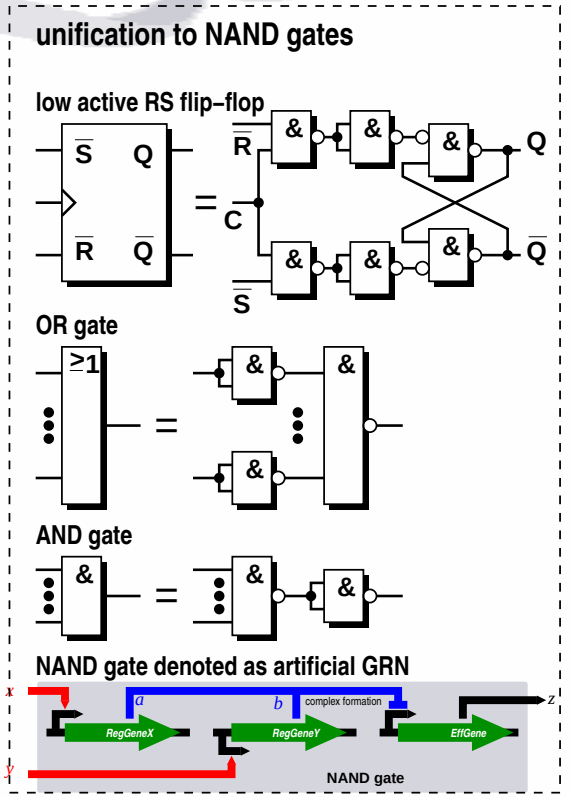
Circuit Construction from Finite Automaton

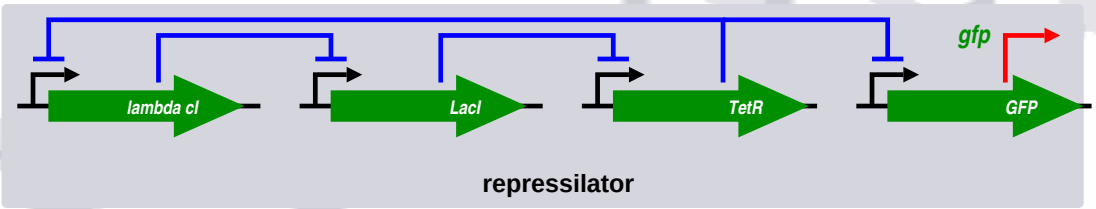




Gray code and Karnaugh optimisation for minimal boolean functions

Artificial GRN from Circuit Description

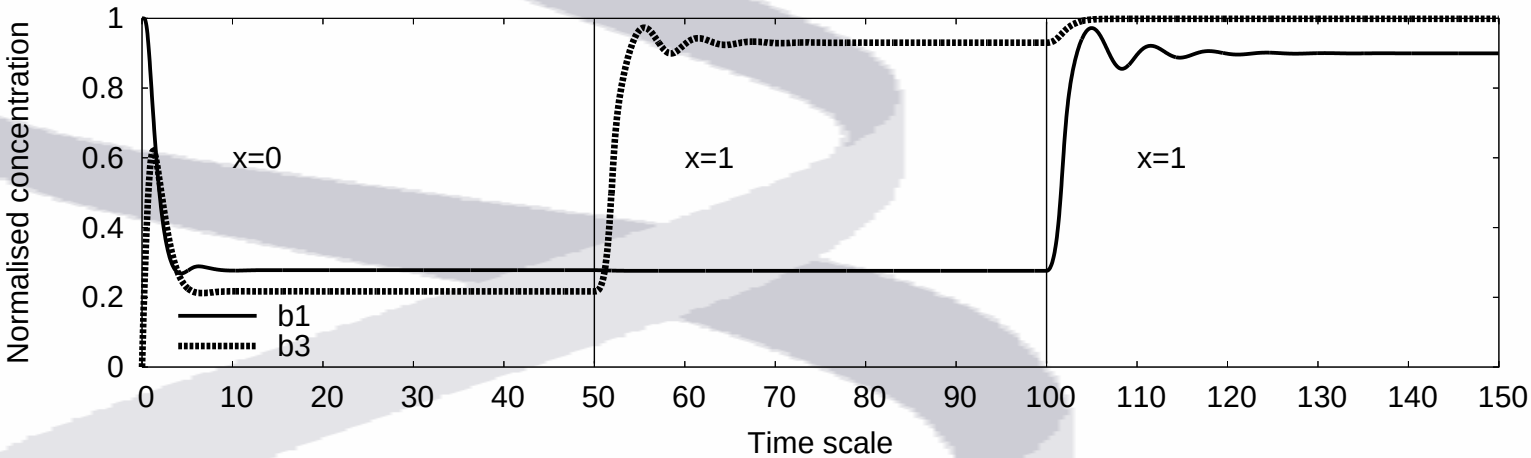
- Clock generator: repressilator GRN (Elowitz et al.)



- ODE derived from Hill kinetics for GRN representing whole circuit (115 regulatory processes)
- Simulation of dynamical behaviour (Copasi)
- Diagram depicts variable bits b_1 and b_3 from path $110 \xrightarrow{0} 010 \xrightarrow{1} 011 \xrightarrow{1} 111$

b_1	b_2	b_3	x	b'_1	b'_2	b'_3
1	1	0	0	0	1	0
0	1	0	1	0	1	1
0	1	1	1	1	1	1

final state reached



Conclusions and Further Work

Conclusions

- GRNs suitable for performing computations
- Definition and composition of computational units
- Presented study as a proof of concept
- Promising simulation results obtained by Hill kinetics
- Adjust parameters to achieve stable/reliable switching behaviour
- Computing agent: complex (artificial) GRN for specific task

Further work

- Coupling of computational units *in vivo*
- Acceleration of GRN-based computations by parallelisation
- Comparison of synthesised artificial GRNs with evolutionary arisen counterparts addressing functional units

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